Palladium-catalyzed amination of electron deficient or relatively rich benzo[*b*]thienylbromides. Preliminary studies of antimicrobial activity and SAR.

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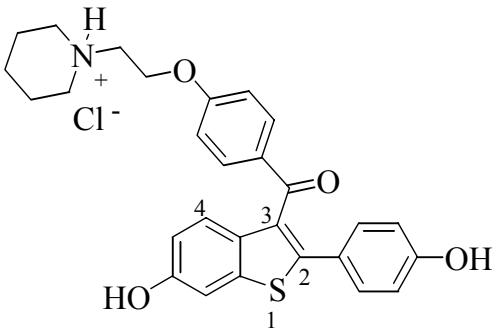
Several diarylamines in the benzo[*b*]thiophene series were prepared by palladium catalyzed amination of ethyl 3-bromobenzo[*b*]thien-2-yl carboxylate with anilines and 5-aminoindole, in good to high yields using $Pd(OAc)_2$, BINAP, Cs_2CO_3 in toluene. The presence of the ester group in the position 2 of the benzo[*b*]thiophene moiety increases the yields and lowers the heating times when compared with reactions using 3-bromobenzo[*b*]thiophene. When aminopyridines, instead of anilines, were used the ligand and the solvent need to be changed to XANTHPHOS

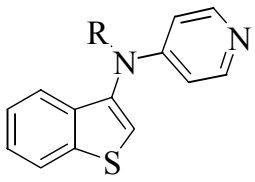
and dioxane in the amination reaction. From 2-aminopyridine a one pot C-N coupling and intramolecular cyclization involving the nitrogen of the pyridine, with lost of ethanol, occurred giving an interesting fluorescent tetracyclic heteroaromatic compound. The antimicrobial activity, the minimal inhibitory concentration (MIC) and structure-activity relationships (SAR) were evaluated. A selectivity with low MICs was observed against *Bacillus Cereus* and good results were also obtained against *Candida albicans*. The acids obtained by hydrolysis of the ester group, as non proteinogenic α,β-unsaturated β-amino acids can be incorporated in a peptide chain to induce conformational constraints.

Introduction

Benzo[*b*]thiophenes are important heterocycles either as biological active molecules or as luminescent components used in organic materials.[1] One of the most important drug based on the benzo[*b*]thiophene system is Raloxifene which was approved by the U.S. Food and Drug Administration for the prevention and treatment of osteoporosis in postmenopausal women.[2] Extensive research is now in course for other potential applications of this drug, namely for the treatment of Alzheimer's disease.[3] In the same series a number of 3-(4-pyridinyl)aminobenzo[*b*]thiophenes, which are selective serotonine re-uptake inhibitors, were prepared and may be useful in the treatment of central nervous system disorders, including obsessive compulsive disorders.[4]



Raloxifene



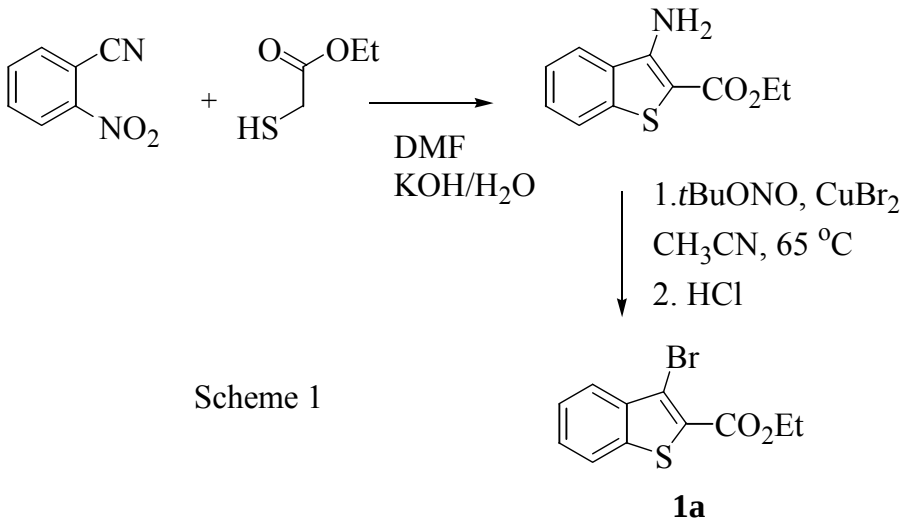
3-(4-pyridinyl)aminobenzo[*b*]thiophenes
R = alkyl

Recently we have been interested in the palladium-catalyzed aryl amination of benzo[*b*]thiophenes either in the benzene or in the thiophene ring to obtain the corresponding diarylamines and in some cases the tetracyclic aromatic compounds resulting from intramolecular cyclizations.[5] We have been able to establish that under the same C-N

coupling[6] conditions [$Pd(OAc)_2$ (3mol%), Cs_2CO_3 (1.4 equiv) and BINAP (4mol%) in toluene] it was possible to obtain either primary amines[5b] or diarylamines[5b,c] in the benzene ring of the benzo[*b*]thiophene moiety. The same conditions were not effective for the amination of 3-bromobenzo[*b*]thiophene with *ortho*-bromoanilines where *t*BuONa, higher amounts of $Pd(OAc)_2$ and BINAP were required.[5c] In this work we extend the scope of the former conditions to the coupling of ethyl 3-bromobenzo[*b*]thien-2-yl carboxylate **1a** (electron-deficient) and 3-bromobenzo[*b*]thiophene **1b** (relatively electron-rich) with aromatic amines. For the coupling with aminopyridines other conditions were required as already described by other authors for heteroaromatic amines.[7] The antimicrobial activity and minimal inhibitory concentration (MIC) of some of the compounds prepared against bacteria and against *Candida albicans* together with structure-activity relationships (SAR) were evaluated. The acids obtained by hydrolysis of the ester group as non-proteinogenic α,β unsaturated β-amino acids can be incorporated into peptides to induce conformational constraints which can help in the study of the structure of proteins.

Results and Discussion

The precursor ethyl 3-bromobenzo[*b*]thien-2-yl carboxylate **1a**, was prepared from the corresponding 3-aminocompound using *t*BuONO and $CuBr_2$.[8] The latter was synthesized by reacting 2-cianonitrobenzene with ethylthioglycolate (Scheme 1).[9]

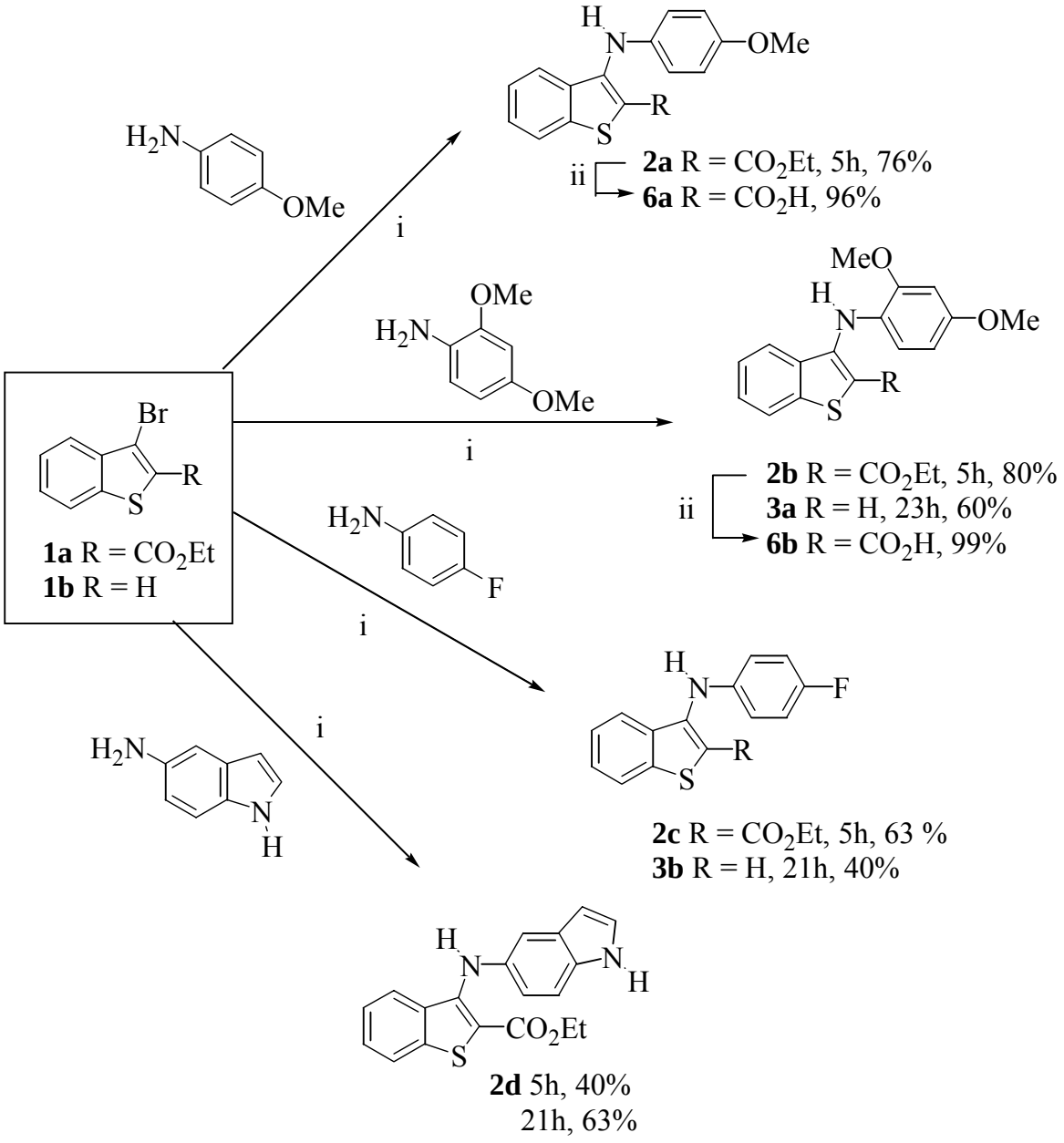


Scheme 1

1a

C-N couplings

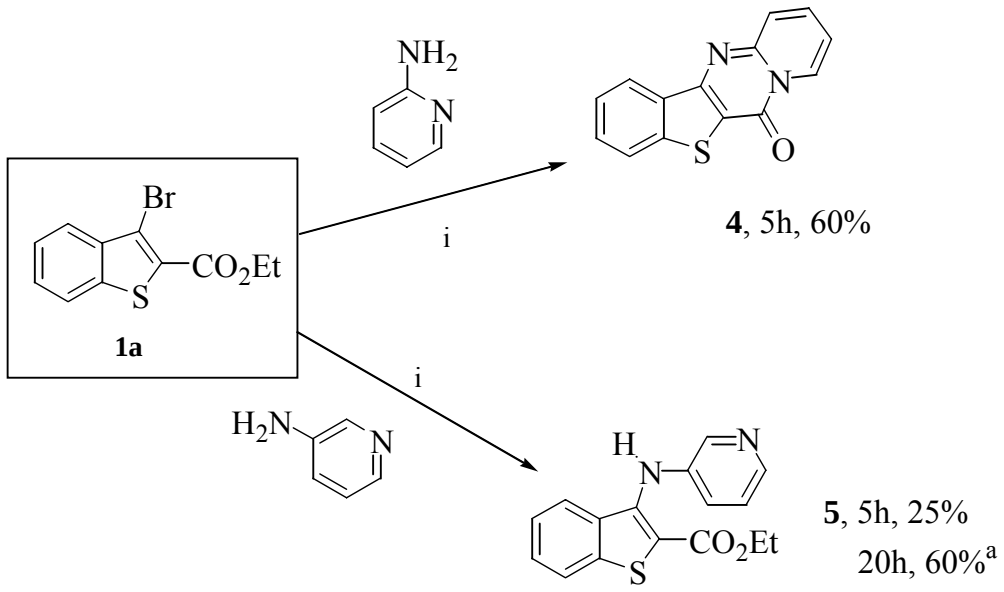
Compound **1a** was coupled with several anilines and with 5-aminoindole in the same conditions to give diarylamines **2** in good to high yields (Scheme 2). When 5-aminoindole was used as coupling component, after 5h of heating the yield of the coupled product was only 40% while leaving the reaction overnight the yield increased to 63%. In order to compare yields, two reactions were performed using 3-bromobenzo[*b*]thiophene **1b**. The presence of the ester group in the 2-position increases the reaction yields and lowers the heating times (Scheme 2).



i) $Pd(OAc)_2$ 3 mol%, BINAP 4 mol% , Cs_2CO_3 1.4 equiv., toluene, 100 ^{o}C, Ar
ii) 1. NaOH 30% EtOH, Δ 2. HCl 1N

Scheme 2

Under the same conditions 2 and 3-aminopyridines didn't react. Changing the conditions to the ones used for the coupling of heteroaromatic amines by other authors[7] afforded the interesting tetracyclic compound **4** from 2-aminopyridine and the diarylamine **5** from 3-aminopyridine (Scheme 3). The former resulted of a C-N coupling followed by an intramolecular cyclization involving the nitrogen atom of the pyridine ring with lost of ethanol. For the synthesis of compound **5** in good yield more time and higher amounts of Pd were needed, $Pd(OAc)_2$ being also effective as Pd source.



i) Pd_2dba_3 (3 mol% Pd), XANTPHOS 4 mol% , Cs_2CO_3 1.4 equiv., dioxane, 100 ^oC, Ar
[a] $Pd(OAc)_2$ (6 mol%)

Scheme 3

Compound **4** as a planar compound can intercalate in the DNA bases acting as an anti-tumor compound and/or as a biomarker due to its fluorescence (Fig.1). It showed a $\lambda_{em(max)}$ at 435 nm and a relative quantum yield of fluorescence in dichloromethane ϕ_{dcm} = 0.13, which was determined by the standard method[11] with 9,10-diphenylanthracene in ethanol as reference (ϕ_{EtOH} = 0.95),[12] using λ_{exc} 368 nm and applying equation 1. This excitation wavelength (λ_{exc}) was chosen from the absorption spectrum of compound **4** (see experimental) after verifying that when λ_{exc} 387 nm was used the compound was already emiting at lower wavelenghts.

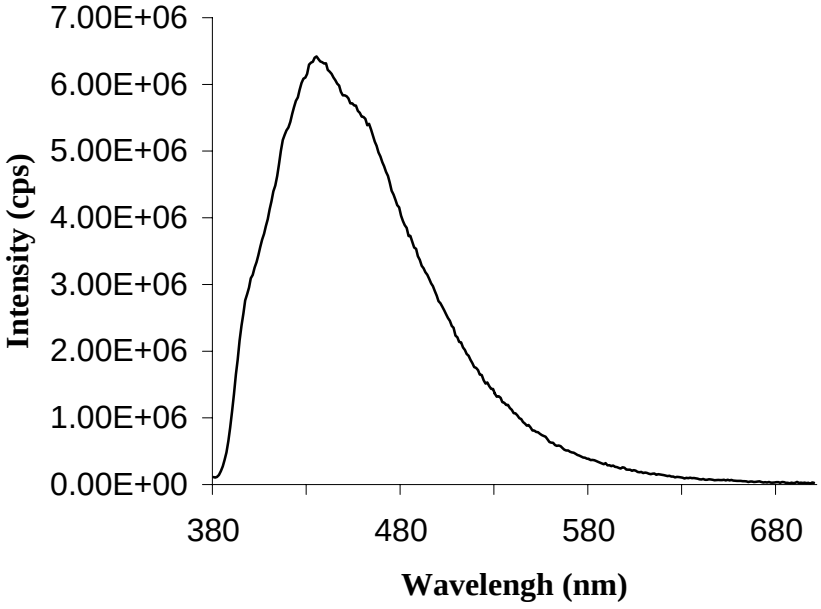


Figure 1. Fluorescence spectrum of compound **4** in dichlorometane (10^{-6} M) (λ_{exc} 368nm) showing a λ_{em} 435 nm.

$$\Phi_S = \frac{A_r F_s n_s^2}{A_s F_r n_r^2} \Phi_r \qquad \text{Equation 1}$$

A- absorbance at the excitation wavelength of the solutions in dcm (s), in EtOH (r).

F - integrated emission area.

n - refraction index of the solvents used.

Subscripts refer to the reference (r) or sample (s) compound.

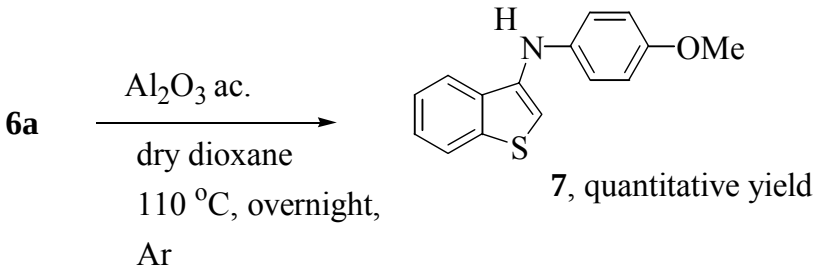
The diarylamine **5** showed only a residual fluorescence (λ_{exc} 362 nm) with a $\lambda_{em\ (max)}$ at 431 nm and a neglectable quantum yield of fluorescence in dichloromethane (ϕ_{dcm} = 0.0008) relatively to the same reference compound in ethanol.

Hydrolysis of the ester group

The corresponding acids **6a** and **6b** were obtained in almost quantitative yield by hydrolysing the diarylamines **2a** and **2b** with NaOH in EtOH/H_2O followed by acidification with HCl (Scheme 2). The β-aminoacids α, β-unsaturated **6a**,**b** thus obtained can be inserted into peptides to induce conformational constraints which can help in the study of the structure of proteins.

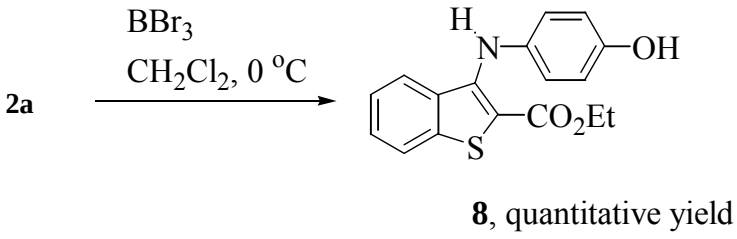
Cyclization attempts of the acids and esters

The acid **6a** was submitted to unsuccessfull attempts of cyclization with PPA and Al_2O_3 acidic in order to obtain the corresponding acridinones. The acids are very polar and they didn't dissolve in PPA even when it was pre-heated. When compound **6b** was melted before addition, decarboxylation to compound **3a** occurred quantitatively with the heat. Using acidic Al_2O_3, which some of us have used as acid catalyst and water retainer in chromenization reactions,[10] in dioxane at 110 ^oC overnight, decarboxylation occurred from **6a** to **7** (Scheme 4). When the reaction was performed at 60 ^oC for 3 days, starting material and compound **7** in a little extent (formed as time passed) were isolated.



Scheme 4

The ester **2a** was submitted to Friedel-Crafts conditions described for the synthesis of cyclic constrained analogues of Raloxifene, resuting from diaryl compounds involving the position 4 of the benzo[*b*]thiophene moiety and possessing an ester group in the 3 position,[3] but in our case only demethylation to the hydroxyl compound **8** occurred (Scheme 5). The same conditions were applied to the fluoro compound **2c** but no reaction took place.



Scheme 5

It seems that the diarylamine moiety doesn't give a good activation for the methods tried in the ester compounds. The easy decarboxylation with the temperature is also a problem for the cyclization of the corresponding acids.

In vitro antimicrobial activity and SAR

A screening of antibacterial activities using two Gram negative (*Escherichia coli* CECT 101 and *Pseudomonas aeruginosa* CECT 108) and two Gram positive bacteria (*Bacillus subtilis* CECT 498 and *Bacillus cereus* CECT 148) and antifungal using *Candida albicans* (CECT 1394) was performed for some of the compounds obtained and the minimal inhibitory concentration (MIC in μg/mL) was determined (Table 1) using an adaptation of agar streak dilution method based on radial diffusion.[13] Suspensions of the microorganism were prepared to contain approximately 10^8 cfu/mL and the plates were inoculated. A stock solution of the synthesized compound (1000 μg/mL) in DMSO was prepared and graded dilutions of the tested compounds were incorporated in a cavity (depth 3mm, diameter 4mm) made in the center of the petridish (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). The plates were incubated at 37 ^oC (for bacteria) and at 30 ^oC (for fungi) for 24h in duplicate. In the same conditions different concentrated solutions of Ampiciline, Chloramphenicol (antibacterial) and Cyclohexamide (antifungal) in DMSO were used as standards. The MIC was considered to be the lowest concentration of the tested compound which inhibits growth of bacteria or fungi on the plate. The diameters of the inhibition zones corresponding to the MICs are presented in Table 1. Positive control using only inoculation and negative control using only DMSO in the cavity were carried out.

The compounds tested were not active against the Gram negative bacteria and *Bacillus subtilis* but only against *Bacillus cereus*. Table 1 shows the MICs for *B.cereus* and *C.albicans* as an evaluation of the antimicrobial activity of the tested compounds.

Table 1. Antimicrobial activity of some of the synthesized compounds.

Compounds	MIC in μg/mL (Zone of inhibition in mm)	
	Bacillus cereus CECT 148	*Candida albicans* CECT 1394
2a	3.13 (14)	100 (8)
8	3.13 (10)	100 (8)
2b	3.13 (11)	100 (5)
6b	3.13 (7)	50 (7)
2c	3.13 (12)	200 (7)
3b	3.13 (14)	50 (8)
4	1.56 (10)	12.5 (10)
5	1.56 (8)	12.5 (8)
Ampiciline	3.13 (13)	_____
Choramphenicol	3.13 (8)	_____
Cyclohexamide	_____	12.5 (5)

CECT-Spanish type culture collection of Valencia University

From the analysis of Table 1 it is possible to conclude that all the compounds exhibit low MICs for *B. cereus* and that compounds containing the pyridine ring **4** and **5** show the lowest MIC (1.56 μg/mL) even lower than the MIC for the antibacterial compounds. For the same compounds the lowest MICs (12.5 μg/mL) were also observed against *C. albicans* and are comparable to Cyclohexamide. The presence of the acid group in compound **6b** lowers the MIC from 100 to 50 μg/mL against *C. albicans* when compared with compound **2b**. Compound **2c** shows the highest MIC against *C. albicans* (200 μg/mL) but without the ester group the corresponding fluoro compound **3b** has a much more lower MIC (50 μg/mL).

Conclusions:

Several diarylamines were prepared using the same C-N coupling conditions from electron deficient or relatively rich benzo[*b*]thienylbromides with aromatic amines. The use of

XANTHPHOS as ligand was needed to couple ethyl 3-bromobenzo[*b*]thien-2-yl carboxylate with aminopyridines. A tetracyclic heteroaromatic fluorescent compound was obtained from the coupling of 2-aminopyridine by a one pot C-N coupling and intramolecular cyclization with lost of ethanol.

Attempts to cyclize the esters and acids to acridinones were unsuccessful so far.

In vitro antimicrobial activity, MICs and SAR were evaluated. A selectivity against *B. cereus* was observed with low MICs. Against *C. Albicans* good results were also obtained especially for the compounds having a pyridine ring.

Experimental Section

General Remarks: Melting points were determined on a Gallenkamp apparatus and are uncorrected. The ^{1}H NMR spectra were measured on a Varian Unity Plus at 300 MHz. Spin-spin decoupling techniques were used to assign the signals. The ^{13}C NMR spectra were measured in the same instrument at 75.4 MHz (using DEPT θ 45º). The IR spectra were recorded as nujol mulls on a Perkin-Elmer 1600-FTIR spectrophotometer. The UV spectra were recorded on a Schimadzu UV-250 1PC, UV-vis recording spectrophotometer.

Elemental analyses were determined on a LECO CHNS 932 elemental analyser. Mass spectra (EI) and HRMS were made by the mass spectrometry service of University of Vigo-Spain or on a Micromass Autospec 3F.

The fluorescence studies were performed on a spectrofluorimeter Spex Fluorolog 1680 Double Spectrometer.

Column chromatography was performed on Macherey-Nagel silica gel 230-400 mesh. Petroleum ether refers to the boiling range 40-60 ^{o}C. Ether refers to diethyl ether. When solvent gradient was used the increase of polarity was done gradually from neat petroleum ether to mixtures of ether/petroleum ether increasing 10% of ether until the isolation of the product.

Ethyl 3-bromobenzo[*b*]thien-2-yl carboxylate 1a: Following the literature methods[8,9] compound **1a** was obtained in 60 % overall yield as an orange solid, m. p. 60-62 ^{o}C . ν_{max} 1725 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 1.45 (t, *J* = 7 Hz, 3H, $CO_2CH_2\mathit{CH_3}$), 4.45 (q, *J* = 7 Hz, 2H, $CO_2\mathit{CH_2}CH_3$), 7.49-7.58 (m, 2H, ArH), 7.80-7.86 (m, 1H, ArH), 7.97-8.02 (m, 1H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.18 (CH_3), 61.77 (CH_2), 114.70 (C), 122.53 (CH), 125.17 (CH), 125. 50 (CH), 127.99 (CH), 138.53 (C), 139.17 (C), 161.34 (C=O) ppm. $C_{11}H_9BrO_2S$ (285.16): calcd. C 46.33, H 3.18, S 11.24; found C 46.69, H 3.56, S 11.48.

General procedure for C-N cross coupling with anilines and 5-aminoindole: A dry Schlenk tube was charged, under Ar, with dry toluene (3-5mL), compound **1a** or **1b**, $Pd(OAc)_2$ (3 mol%), *rac*BINAP (4mol%), Cs_2CO_3 (1.4equiv). and at the end the amine. The reaction was stirred and heated at 100 ^{o}C for several hours (Scheme 2). After cooling water and ether were added and the phases were separated. The aqueous phase was extracted with more ether and the organic phases were collected, dried ($MgSO_4$), filtered and the removal of the solvent gave an oil, after addition of MeOH to remove traces of toluene. The oil was submitted to column chromagraphy.

Ethyl 3-[(4-methoxyphenyl)amino]benzo[*b*]thien-2-yl carboxylate 2a: From compound **1a** (300 mg, 1.05 mmol), *p*-anisidine (130 mg, 1.05 mmol) and column chromatography using solvent gradient from neat petroleum ether to 10% ether/petroleum ether, product **2a** (263 mg, 76%) was obtained as an orange solid. Recrystallization from ether/petroleum ether gave yellow crystals, mp 87-89 ºC. ν_{max} 3305 (NH), 1667 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.42 (t, *J* = 7 Hz, 3H, $CO_2CH_2CH_3$), 3.83 (s, 3H, OCH_3), 4.38 (q, *J* = 7 Hz, 2H, $CO_2CH_2CH_3$), 6.86 (d, *J* = 8.7 Hz, 2H, 2xArH), 7.06-7.11 (m, 3H, ArH), 7.22 (d, *J* = 8 Hz, 1H, ArH), 7.35-7.40 (1H, m, Ar-H), 7.73 (d, *J* = 8 Hz, 1H, ArH), 8.81 (s, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.44 (CH_3), 55.46 (OCH_3), 60.69 (CH_2), 103.77 (C), 114.22 (2xCH), 123.15 (CH), 123.18 (CH), 124.90 (2xCH), 125.59 (CH), 127.45 (CH), 131.58 (C), 135.01 (C), 140.23 (C), 147.60 (C), 156.69 (C), 165.74 (C=O) ppm. $C_{18}H_{17}NO_3S$ (327.40): calcd. C 66.04, H 5.23, N 4.28, S 9.79; found C 66.00, H 5.43, N 4.48, S 9.79.

Ethyl 3-[(2,4-dimethoxyphenyl)amino]benzo[*b*]thien-2-yl carboxylate 2b: From compound **1a** (300 mg, 1.05 mmol), 2,4-dimethoxyaniline (161 mg, 1.05 mmol) and column chromatography using solvent gradient from neat petroleum ether to 20% ether/petroleum ether, product **2b** (300 mg, 80%) was obtained as an orange solid. Recrystallization from ether/petroleum ether gave yellow crystals, mp 95-96 ºC. ν_{max} 3319 (NH), 1666 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.42 (t, *J* = 7 Hz, 3H, $CO_2CH_2CH_3$), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.38 (q, *J* = 7 Hz, 2H, $CO_2CH_2CH_3$), 6.37 (dd, *J* = 8.7 and 2.7 Hz, 1H, 5'-H), 6.56 (d, *J* = 2.7 Hz, 1H, 3'-H), 6.93 (d, *J* = 8.7 Hz, 1H, 6'-H), 7.08-7.14 (m, 1H, ArH), 7.34-7.41 (m, 2H, ArH), 7.72 (d, *J* = 8 Hz, 1H, ArH), 8.58 (s, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.45 (CH_3), 55.55 (OCH_3), 55.69 (OCH_3), 60.66 (CH_2), 99.21 (CH), 103.41 (CH) 104.34 (C), 123.12 (CH), 123.16 (CH), 123.17 (CH), 124.30 (C), 125.35 (CH), 127.40 (CH), 131.91 (C), 140.06 (C), 147.08 (C), 153.19 (C), 157.16 (C), 165.55 (C=O) ppm. $C_{19}H_{19}NO_4S$ (357.42): calcd. C 63.85, H 5.36, N 3.92, S 8.97; found C 63.68, H 5.49, N 4.04, S 8.96.

Ethyl 3-[(4-fluorophenyl)amino]benzo[*b*]thien-2-yl carboxylate 2c: From compound **1a** (150 mg, 0.526 mmol), 4-fluoroaniline (64.0 mg, 0.526 mmol) and column chromatography using solvent gradient from neat petroleum ether to 20% ether/petroleum ether, product **2c** (105 mg, 63%) was obtained as a yellow solid. Recrystallization from ether/petroleum ether gave light yellow crystals, mp 119-121 ºC. ν_{max} 3294 (NH), 1668 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.42 (t, *J* = 7 Hz, 3H, $CO_2CH_2CH_3$), 4.39 (q, *J* = 7 Hz, 2H, $CO_2CH_2CH_3$), 6.97-7.17 (m, 5H, ArH), 7.25-7.29 (m, 1H, ArH), 7.38-7.44 (m, 1H, ArH), 7.76 (d, *J* = 8 Hz, 1H, ArH), 8.75 (s, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.40 (CH_3), 60.90 (CH_2), 106.19 (C) 115.73 (d, *J* = 22.7 Hz, 3' and 5'-CH), 123.27 (CH), 123.39 (CH), 123.80 (d, *J* = 8 Hz, 2' and 6'-CH), 125.37 (CH), 127.57 (CH), 131.60 (C), 138.27 (d, *J* = 2.8 Hz, 1'-C), 140.09 (C), 146.41 (C), 159.45 (d, *J* = 242.7 Hz, CF), 165.55 (C=O) ppm. $C_{17}H_{14}FNO_2S$ (315.36): calcd. C 64.75, H 4.47, N 4.44, S 10.17; found C 64.40, H 4.77, N 4.37, S 10.36.

Ethyl 3-[(5-indole)amino]benzo[*b*]thien-2-yl carboxylate 2d: From compound **1a** (250 mg, 0.877 mmol), 5-aminoindole (116 mg, 0.877 mmol), heating for 21h and column chromatography using solvent gradient from neat petroleum ether to 50% ether/petroleum ether, product **2d** (155 mg, 63%) was obtained as an orange solid. Recrystallization from ether/petroleum ether gave yellow crystals, mp 166-168 ºC. ν_{max} 3382 (NH), 3307 (NH), 1631 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.43 (t, *J* = 7 Hz, 3H, $CO_2CH_2CH_3$), 4.39 (q, *J* = 7 Hz, 2H, $CO_2CH_2CH_3$), 6.49-6.51 (m, 1H, ArH), 6.95-7.00 (m, 1H, ArH), 7.07 (dd , *J* = 8.4 and 2.1 Hz, 1H, 6'-H), 7.14 (d, *J* = 8 Hz, 1H, ArH) 7.25-7.27 (m, 1H, ArH), 7.30-7.34 (m, 2H, ArH), 7.46 (d, *J* = 2.1 Hz, 1H, 4'-H), 7.72 (d, *J* = 8 Hz, 1H, ArH),

8.20 (s, 1H, indole-NH) 9.01 (s, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.48 (CH_3), 60.59 (CH_2), 102.56 (C) 102.72 (CH), 111.30 (CH) 115.90 (CH), 119.91 (CH) 123.03 (CH), 123.04 (CH), 125.17 (CH), 125.91 (CH), 127.39 (CH), 128.17 (C), 131.79 (C), 133.58 (C), 134.49 (C) 140.32 (C), 148.52 (C), 165.89 (C=O) ppm. $C_{19}H_{16}N_2O_2S$ (336.41): calcd. C 67.84, H 4.79, N 8.33, S 9.53; found C 67.56, H 4.90, N 8.29, S 9.32.

3-[(2,4-Dimethoxyphenyl)amino]benzo[*b*]thiophene 3a: From compound **1b** (249 mg, 1.17 mmol), 2,4-dimethoxyaniline (180 mg, 1.17 mmol) and column chromatography using solvent gradient from neat petroleum ether to 10% ether/petroleum ether, product **3a** (200 mg, 60%) was obtained as an oil. ^{1}H NMR ($CDCl_3$): δ = 3.83 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.10 (s, 1H, NH), 6.50 (dd, *J* = 8.7 and 2.7 Hz, 1H, 5'-H), 6.64 (d, *J* = 2.7 Hz, 1H, 3'-H), 6.90 (s, 1H, 2-H), 7.22 (d, *J* = 8.7 Hz, 1H, 6'-H), 7.41-7.44 (m, 2H, 7 and 4-H), 7.76-7.80 (m, 1H, ArH), 7.86-7.90 (m, 1H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 55.53 (OCH_3), 55.56 (OCH_3), 99.24 (CH), 103.74 (CH) 104.11 (C), 116.08 (CH), 120.19 (CH), 123.01 (CH), 123.61 (CH), 124.67 (CH), 127.40 (C), 134.27 (C), 136.08 (C), 138.73 (C), 149.09 (C), 153.96 (C) ppm. MS m/z (%) 287 (M^++2, 7), 286 (M^++1, 20), 285 (M^+, 100), 270 (M^+-15, 51), 138 (19). HRMS M^+ $C_{16}H_{13}NO_2S$: calcd. 285.082298; found 285.082351.

3-[(4-Fluorophenyl)amino]benzo[*b*]thiophene 3b: From compound **1b** (300 mg, 1.41 mmol), 4-fluoroaniline (156 mg, 1.41 mmol) and column chromatography using solvent gradient from neat petroleum ether to 10% ether/petroleum ether, product **3b** (127 mg, 40%) was obtained as a white solid. Recrystallization from ether/petroleum ether gave colourless crystals, mp 87-89 ºC. ν_{max} 3388 (NH) cm^{-1}.^{1}H NMR ($CDCl_3$): δ = 5.68 (s, 1H, NH), 6.91 (s, 1H, 2-H), 6.98 (s, 4H, 2', 3', 5' and 6'-H), 7.38-7.41 (m, 2H, ArH), 7.64-7.67 (m, 1H, ArH), 7.83-7.86 (m, 1H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 107.83 (CH), 115.90 (d, *J* = 22.5 Hz, 3' and 5'-CH), 118.05 (d, *J* = 8 Hz, 2' and 6'-CH), 120.41 (CH), 123.25 (CH), 123.91 (CH), 124.92 (CH), 134.20 (C), 135.77 (C) 138.97 (C), 140.64 (C), 157.37 (d, *J* = 238.7 Hz, CF) ppm. $C_{17}H_{10}FNS$ (243.30): calcd. C 69.11, H 4.14, N 5.76, S 13.18; found C 69.00, H 4.35, N 5.76, S 13.05.

General procedure for C-N cross coupling with aminopyridines: A dry Schlenk tube was charged, under Ar, with dry 1,4-dioxane (3-5mL), compound **1a**, Pd_2dba_3 (3mol%Pd) or $Pd(OAc)_2$ (6 mol%), XANTHOPS (4mol%), Cs_2CO_3 (1.4equiv). and at the end the aminopyridine (1.1 equiv). The reaction mixture was stirred and heated at 100 ^{o}C for several hours (Scheme 3). After cooling water and ether were added and the phases were separated. The aqueous phase was extracted with more ether and the organic phases were collected, dried ($MgSO_4$), filtered and removal of solvent gave an oil or a solid. The oil was submitted to column chromagraphy.

6-Oxo-6*H*-benzo[*b*]thieno[3,2-*d*]pyrido[1,2-*a*]pyrimidine 4: From compound **1a** (200 mg, 0.702 mmol), 2-aminopyridine (73.0 mg, 0.772 mmol) product **4** was obtained as a beije solid (100 mg, 60%). Recrystallization from ether gave light beije crystals, mp 211-213 ºC. ν_{max} 1693 (C=O) cm^{-1}. λ_{max} (CH_2Cl_2) (ε $dm^3mol^{-1}cm^{-1}$) 387 (6066), 368 (7238), 303 (2062), 276 (14842), 257 (18772) nm. ^{1}H NMR (DMSO-d_6): δ = 7.31-7.38 (m, 1H, ArH), 7.58-765 (m, 1H, ArH), 7.69-7.76 (m, 1H, Ar-H) 7.81-7.93 (m, 2H, ArH), 8.16 (d, *J* = 8 Hz, 1H, ArH), 8.37 (broad d, *J* = 8 Hz, 1H, ArH) 9.01 (broad d, *J* = 8 Hz, 1H, ArH) ppm. ^{13}C NMR (DMSO-d_6): δ = 113.21 (C), 115.46 (CH), 123.80 (CH), 124.01 (CH), 125.52 (CH), 125.91 (CH), 126.32 (CH), 130.06 (CH), 133.93 (C), 135.98 (CH), 141.23 (C), 149.28 (C), 153.38 (C), 154.05 (C) ppm. MS (EI) m/z (%) 252 (M^+, 100), 224 (M^+-CO, 35). HRMS M^+ $C_{15}H_{11}N_2OS$: calcd. 252.035735; found 252.035591.

Ethyl 3-(3-aminopyridine)benzo[*b*]thien-2-yl carboxylate 5: From compound **1a** (200 mg, 0.702 mmol), 3-aminopyridine (73.0 mg, 0.772 mmol) and column chromatography using solvent gradient from neat petroleum ether to neat ether, product **5** (127 mg, 40%) was obtained as an orange solid. Recrystallization from ether/petroleum ether gave yellow light crystals, mp 112-114 ºC. ν_{max} 3293 (NH), 1661 (C=O) cm^{-1}. λ_{max} (CH_2Cl_2) (ε $dm^3mol^{-1}cm^{-1}$) 362 (12099), 263 (22764) 225 (13469) nm. ^{1}H NMR ($CDCl_3$): δ = 1.43 (t, *J* = 7 Hz, 3H, $CO_2CH_2CH_3$), 4.40 (q, *J* = 7 Hz, 2H, $CO_2CH_2CH_3$), 7.18-7.24 (m, 2H, ArH), 7.29-7.38 (m, 2H, ArH), 7.42-7.48 (m, 1H, ArH), 7.79-7.71(d, , *J* = 8.1 Hz, 1H, ArH), 8.33 (broad d, *J* = 4.5 Hz, 1H, Ar-H), 8.44 (d, *J* = 2.4 Hz, 1H, 2'-H), 8.70 (s, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.35 (CH_3), 61.17 (CH_2), 109.34 (C), 123.41(2xCH), 123.83 (CH), 125.00 (CH), 127.28 (CH), 127.74 (CH), 131.59 (C), 139.01 (C), 139.87 (C), 142.79 (CH), 144.10 (CH), 144.36 (C), 165.26 (C=O) ppm. $C_{16}H_{14}O_2N_2S$ (298.34): calcd. C 64.41, H 4.73, N 9.39, S 10.75; found C 64.09, H 4.95, N 9.24, S 10.70.

General procedure for the hydrolysis of the ester group: Compounds **2a** or **2b** in ethanol (10-20 mL) were treated with a NaOH 30% aqueous solution (5 equiv.). The mixture was stirred and heated to reflux using a water bath for some hours following the reaction by TLC. After cooling the ethanol was evaporated under reduced pressure and to the solid obtained water was added and then HCl 1N till the precipitation of the acids **6a** or **6b** which were obtained by filtration and drying at 50 ºC.

3-[(4-Dimethoxyphenyl)amino]benzo[*b*]thien-2-yl carboxylic acid 6a: From compound **2a** (150 mg, 0.459 mmol), product **6a** (131 mg, 96%) was obtained as yellow solid. Recrystallization from ether gave yellow crystals, mp 148-150 ºC. ν_{max} 3300 (NH), 1642 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 3.85 (s, 3H, OCH_3), 6.89 (d, *J* = 9 Hz, 2H, 2xArH), 7.05 -7.19 (m, 4H, ArH), 7.40 (broad t, *J* = 8 Hz, 1H, ArH), 7.53 (d, *J* = 8 Hz, 1H, ArH), 8.84 (s, 1H, NH) ppm. $C_{16}H_{13}NO_3S$ (299.35): calcd. C 64.20, H 4.38, N 4.69, S 10.71; found C 64.03, H 4.59, N 4.66, S 10.66.

3-[(2,4-Dimethoxyphenyl)amino]benzo[*b*]thien-2-yl carboxylic acid 6b: From compound **2b** (360 mg, 1.01 mmol), product **6b** (328 mg, 99%) was obtained as yellow solid. Recrystallization from ether gave yellow crystals, mp 152-154 ºC. ν_{max} 3354 (NH), 1688 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 3.76 (s, 6H, 2xOCH_3), 6.45 (dd, *J* = 8.7 and 3 Hz, 1H, 5'-H), 6.68 (d, *J* = 3 Hz, 1H, 3'-H), 6.89 (d, *J* = 8.7 Hz, 1H, 6'-H), 7.13-7.17 (m, 2H, ArH), 7.40-7.45 (m, 1H, ArH), 7.88 (d, *J* = 8.1 Hz, 1H, ArH), 8.53 (s, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ = 55.42 (OCH_3), 55.76 (OCH_3), 99.45 (CH), 103.75 (C) 104.24 (CH), 123.21(C), 123.63 (CH), 123.67(CH), 123.79 (CH), 124.42 (CH), 127.73 (CH), 131.52 (C), 139.08(C), 146.47 (C), 153.33 (C), 157.33 (C), 166.51 (C=O) ppm. $C_{17}H_{15}NO_4S$ (329.37): calcd. C 61.99, H 4.59, N 4.25, S 9.74; found C 61.99, H 4.83, N 4.31, S 9.60.

Cyclization attempts:

3-[(4-Methoxyphenyl)amino]benzo[*b*]thiophene 7: To a solution of compound **6a** (50.0 mg, 0.190 mmol) in dry CH_2Cl_2 (3mL) in a Schlenk tube, Al_2O_3 acidic Brockman I (10 equiv.) was added and the suspension was left stirring overnight at 110 ºC under Ar. After cooling the alumina was removed by filtration and the solvent removal gave product **7** (46 mg, quantitative yield) as a solid m.p. 102-104 ºC. ^{1}H NMR ($CDCl_3$): δ = 3.81(s, 3H, OCH_3), 5.70 (broad s, 1H, NH), 6.76 (s, 1H, 2-H), 6.87 (d, *J* = 9 Hz, 2H, 2xArH), 7.05 (d, *J* = 9 Hz, 2H, 2xArH), 7.35-7.42 (m, 2H, ArH), 7.64-7.69 (m, 1H, ArH), 7.81-7.85 (m, 1H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 55.62 (OCH_3), 104.60 (CH), 114.74 (2xCH), 119.44 (2xCH), 120.19 (CH), 123.20 (CH), 123.74 (CH), 124.79 (CH), 133.95 (C), 136.95

(C), 137.59 (C), 139.01 (C), 154.42 (C) ppm. MS (EI) m/z (%) 255 (M^+, 80), 240 (M^+-Me, 100). HRMS M^+ $C_{15}H_{13}NOS$: calcd. 255.0718; found 255.0726.

Ethyl 3-[(4-hydroxyphenyl)amino]benzo[*b*]thien-2-yl carboxylate 8: To a solution of compound **2a** (100 mg, 0.301 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C it was added a solution of BBr_3 in CH_2Cl_2 1M (1.6 mmol, 1.6 mL). The solution went green and it was left stirring at 0 °C for 2h. A saturated solution of $NaHCO_3$ was added slowly and the extractions were done using CH_2Cl_2. The organic phases were collected, dried, filtered and removal of solvent gave a yellow green solid (95.0 mg, quantitative yield). Recrystallization from ether/petroleum ether gave yellow green crystals, mp 152-154 °C. ν_{max} 3330 (NH), 1630 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.41 (t, *J* = 7 Hz, 3H, $CO_2CH_2CH_3$), 4.39 (q, *J* = 7 Hz, 2H, $CO_2CH_2CH_3$), 4.90 (broad s, 1H, OH), 6.81 (d, *J* = 8.7 Hz, 2H, 2xArH), 7.01-7.20 (m, 3H, ArH), 7.2 (d, *J* = 8.2 Hz, 1H, ArH), 7.35 (broad t, *J* = 8.2 Hz, 1H, ArH), 7.72 (d, *J* = 8.2 Hz, 1H, ArH), 8.77 (s, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.47 (CH_3), 60.78 (CH_2), 115.83 (CH), 123.22 (4xCH), 123.25 (CH), 125.17 (CH), 125.64 (CH), 127.52 (CH), 131.63 (C), 135.24 (C), 140.32 (C), 147.56 (C), 147.57 (C) 152.71 (C), 165.84 (C=O) ppm. MS (EI) m/z (%) 313 (M^+, 50), 311 (M^+-2, 90), 267 (100), 239 (80) 210 (90). HRMS M^+ $C_{17}H_{15}NO_3S$: calcd. 313.0773; found 313.0760.

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